1,4-DIAMINO-2-NITROBENZENE
(2-Nitro-*para*-phenylenediamine)

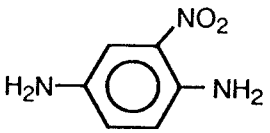
This substance was considered by a previous Working Group, in 1977 (IARC, 1978a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Serv. Reg. No.: 5307-14-2
Chem. Abstr. Name: 2-Nitro-1,4-benzenediamine
IUPAC Systematic Name: 2-Nitro-*para*-phenylenediamine
Colour Index No.: 76070
Synonyms: 4-Amino-2-nitroaniline; CI Oxidation Base 22; 2,5-diaminonitrobenzene; 2-nitro-4-aminoaniline; 2-nitro-1,4-benzenediamine; 2-nitro-1,4-diaminobenzene; 2-nitro-1,4-phenylenediamine; nitro-*para*-phenylenediamine; *ortho*-nitro-*para*-phenylenediamine; NPD



$C_6H_7N_3O_2$ Mol. wt: 153.14

1.1.2 *Chemical and physical properties of the substance*

(*a*) *Description*: Reddish-brown crystalline powder, with a greenish cast (Cosmetic Ingredient Review Expert Panel, 1985)

(*b*) *Melting-point*: 137–140 °C (95–99% pure) (Janssen Chimica, 1990; Aldrich Chemical Co., 1992); 142–144 °C (97.5–100% pure) (Jos. H. Lowenstein & Sons, 1991)

(*c*) *Spectroscopy data*: Infrared, ultraviolet and nuclear magnetic resonance spectral data have been reported (Sadtler Research Laboratories, 1980; Pouchert, 1981, 1983; Sadtler Research Laboratories, 1991).

(*d*) *Solubility*: Slightly soluble in water (0.18% w/w), ethanol, polar organic compounds and benzene; soluble in acetone and diethyl ether (Marzulli *et al.*, 1981)

(*e*) *Octanol/water partition coefficient (P)*: log P, 3.7 (Cosmetic Ingredient Review Expert Panel, 1985)

1.1.3 *Trade names, technical products and impurities*

Some trade names are Durafur Brown 2R; Fouramine 2R; Fourrine 36; Fourrine Brown 2R; Ursol Brown RR; Zoba Brown RR.

1,4-Diamino-2-nitrobenzene is available commercially in purities ranging from 95 to 100%, with 4-amino-3-nitroacetanilide as a possible impurity (from incomplete hydrolysis of the acetylated intermediate; Cosmetic Ingredient Review Expert Panel, 1985). It has the following specifications: ash, 0.1% (max.); iron, 40 ppm (mg/kg) (max.); lead (see IARC, 1980a, 1987a), 5 ppm (mg/kg) (max.); and arsenic (see IARC, 1980b, 1987b), 2 ppm (mg/kg) (max.). It is also available in research quantities at purities ranging from 90 to 99% (Janssen Chimica, 1990; Jos. H. Lowenstein & Sons, 1991; Aldrich Chemical Co., 1992; Fluka Chemie AG, 1993).

1.1.4 *Analysis*

Qualitative and quantitative determinations of 1,4-diamino-2-nitrobenzene and its derivatives are made using paper chromatography, high-performance liquid chromatography, reverse-phase liquid chromatography and thin-layer chromatography and by spectrophotometric methods and electrophoresis (Cosmetic Ingredient Review Expert Panel, 1985).

1.2 Production and use

1.2.1 *Production*

1,4-Diamino-2-nitrobenzene was first synthesized in 1907 (Society of Dyers and Colourists, 1971a). It is prepared by the reaction of 1,4-diaminobenzene (*para*-phenylenediamine; see IARC, 1978b) with acetic anhydride to form 1,4-bis(acetylamino)benzene, which is nitrated and hydrolysed (Cosmetic Ingredient Review Expert Panel, 1985).

Approximately 150 kg of 1,4-diamino-2-nitrobenzene are used in hair colouring products in the USA annually, according to industry estimates. It is produced by one company each in France and Germany (Chemical Information Services, 1991).

1.2.2 *Use*

1,4-Diamino-2-nitrobenzene is used as a dye in semi-permanent hair colouring products. These products are generally shampooed into the hair, lathered and then allowed to remain in contact with the hair and scalp for 30–45 min. (US National Cancer Institute, 1979; Cosmetic Ingredient Review Expert Panel, 1985).

1,4-Diamino-2-nitrobenzene is also used as an ingredient in permanent hair dye formulations, at levels of up to about 1% (Cosmetic Ingredient Review Expert Panel, 1985).

The active ingredient in these dyes reacts in an oxidative coupling reaction with hydrogen peroxide within the hair shafts to produce the permanent colours. 1,4-Diamino-2-nitrobenzene is used to produce light-brown and reddish shades (US National Cancer Institute, 1979). In a similar process, it is used in fur dyeing to produce a red-brown colour, or to add red shading when used in combination with other oxidation bases (Society of Dyers and Colourists, 1971b; US National Cancer Institute, 1979).

1.3 Occurrence

1.3.1 *Natural occurrence*

1,4-Diamino-2-nitrobenzene is not known to occur as a natural product.

1.3.2 *Occupational exposure*

No data were available to the Working Group.

On the basis of a survey conducted in the USA between 1981 and 1983, the US National Institute for Occupational Safety and Health estimated that a total of 29 422 workers, including 23 531 women, may have been exposed to 1,4-diamino-2-nitrobenzene in 3160 facilities (US National Library of Medicine, 1992).

1.4 Regulations and guidelines

The use of 1,4-diamino-2-nitrobenzene as a hair dye is not permitted in Italy or Denmark (Liebscher & Spengler, 1989).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were fed diets containing 2200 or 4400 mg/kg of diet (ppm) 1,4-diamino-2-nitrobenzene (commercial grade; melting-point, 138–139 °C [purity unspecified]) for 78 weeks, followed by a 12-week (males and low-dose females) or 13-week (high-dose females) observation period before sacrifice. Control groups of 20 males and 20 females were maintained on basal diet for up to 90 weeks. Dose-related mean body weight depression (15–20%) was observed in both males and females throughout the experiment. Survival rates, analysed by Tarone's test, did not differ significantly among males or females. Survival at the end of the study was: males, 18/20 (controls), 46/50 (low-dose), 49/50 (high-dose); females, 20/20 (controls), 45/50 (low-dose),

43/50 (high-dose). In females, the incidences of hepatocellular adenomas were: control, 1/20; low-dose, 10/49; and high-dose, 14/48 [p = 0.01, Cochran Armitage test for trend]; three hepatocarcinomas occurred in high-dose females. No difference in the incidence of hepatocellular tumours was observed in males, and there was no increase in the incidence of other tumours in either sex (US National Cancer Institute, 1979). The histopathological findings in the livers of female mice in this study were confirmed by Reznik and Ward (1979).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six weeks of age, were fed diets containing 550 or 1100 mg/kg of diet (ppm) (males) and 1100 or 2200 ppm (females) 1,4-diamino-2-nitrobenzene (commercial grade [purity unspecified]) for 78 weeks, followed by a 27-week observation period before all surviving animals were killed. Control groups of 20 males and 20 females were maintained on basal diet for up to 105 weeks. A dose-related mean body weight depression of approximately 10% was apparent in male rats from week 12 until week 87. Female rats had a dose-related mean body weight depression (> 10%) throughout the study. Survival rates, analysed by Tarone's test, were not significantly different between treated and control animals of either sex. Survivors at the end of the study were: males, 16/20 (controls), 46/50 (low-dose), 47/50 (high-dose); females, 18/20 (controls), 45/50 (low-dose), 38/50 (high-dose). No significant increase in the incidence of tumours was observed (US National Cancer Institute, 1979).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

About 85 g of a commercial semi-permanent hair dye formulation containing 1.36% 1,4-diamino-2-nitrobenzene enriched with ^{14}C-labelled compound at 0.576 μCi/mg was applied on two occasions to the hair of human volunteers, worked in gently for 5–8 min and allowed to remain in contact with the hair and scalp for an additional 30 min. On the first occasion, the hair was clipped, and radiolabel accounting for 0.14% of that applied was detected in the urine over a seven-day period; half was excreted in the urine after 24 h. On the second occasion, the hair was clipped only after 30 days: cumulative absorption was 0.19% on the first day and 0.75% on the 30th day; half of the radiolabel was excreted after 150 h. Urinary metabolites were not identified (Wolfram & Maibach, 1985).

4.1.2 *Experimental systems*

The same commercial hair dye formulation as used above was applied to the scalp hair of rhesus monkeys and allowed to remain in contact for 30 min. Radiolabel accounting for 0.55% of that applied was detected in urine over a seven-day period; half was excreted in the urine after 24 h. Urinary metabolites were not identified (Wolfram & Maibach, 1985).

^{14}C-1,4-Diamino-2-nitrobenzene (1.32 mci/mmol [8.6 μCi/mg]) in acetone was applied to the forearms of adult rhesus monkeys of each sex and to the backs of immature

Pitman-Moore white swine (4 μg/cm^2). The skin contact area ranged from 3 to 15 cm^2. Skin penetration over the 24-h exposure period was 29.9% of the applied dose in monkeys and 17.7% in swine. The peak rate of excretion of radiolabel in urine occurred between 4 and 8 h in monkeys and between 8 and 12 h in swine. Urinary metabolites were not identified (Marzulli *et al.*, 1981).

Male Sprague-Dawley rats were injected intraperitoneally or intravenously with ^{14}C-1,4-diamino-2-nitrobenzene (6.2 mCi/mmol [6.5 μCi/mg]; radiochemical purity, > 99%) in isotonic buffer solution (pH 7.4) at a dose of 2.6 mg/kg bw. After intraperitoneal injection, 37.4% of the radiolabel was excreted in the urine and 54.3% in the faeces within 24 h; total excretion over four days amounted to 96% of the dose. Within 24 h after intravenous injection to cannulated rats, 42.2% of the radiolabel was excreted in the bile, 34.5% in urine, 8.1% in faeces and 0.65% in the digestive tract. The highest concentration of radiolabel was found at 1 h, except in the small and large intestines where it was found after 3 h, followed by a rapid decrease in concentration. Only small amounts of radiolabel were present in tissues after 48 h. After intraperitoneal injection, the urinary metabolites identified were N^1,N^4-diacetyl-1,2,4-triaminobenzene (N^1,N^4-diacetyl-2-amine-*p*-phenylenediamine), representing 13.4% of the urinary radiolabel, and N^4-acetyl-1,4-diamino-2-nitrobenzene (N^4-acetyl-2-nitro-*p*-phenylenediamine), representing 5.8%. Evidence was obtained for the presence of conjugates of unstable metabolites (Nakao & Takeda, 1983).

In an extension of the previous study, the *N*-acetylation reaction following intraperitoneal administration of 1,4-diamino-2-nitrobenzene, 1,2,4-triaminobenzene or various *N*-acetylated metabolites was examined in male Sprague-Dawley rats given injections of 30 or 100 mg/kg bw in a 2% carboxymethyl cellulose sodium salt solution. 1,4-Diamino-2-nitrobenzene was metabolized to N^4-acetyl-1,4-diamino-2-nitrobenzene, N^4-acetyl-1,2,4-triaminobenzene and N^1,N^4-diacetyl-1,2,4-triaminobenzene by regioselective N^4-acetylation and subsequent nitroreduction, followed by regioselective N^1-acetylation (Nakao *et al.*, 1987).

1,2,4-Triaminobenzene and its N^4-acetyl derivative were shown to be intermediates in the anaerobic metabolism of 1,4-diamino-2-nitrobenzene and N^4-acetyl-1,2-diamino-2-nitrobenzene in liver microsomes and cytosol from male Sprague-Dawley rats. The cytosolic nitro-reducing activity was attributed to xanthine oxidase, aldehyde oxidase and, possibly, other unknown enzymes (Nakao *et al.*, 1991).

4.2 Toxic effects

4.2.1 *Humans*

A case of psoriasis-like contact dermatitis was reported following use of a semi-permanent hair dye containing 1,4-diamino-2-nitrobenzene; a patch test carried out with 1% of the compound was positive (Perno & Lisi, 1990).

4.2.2 *Experimental systems*

In a study of the transformation of lymphocytes into blastocytes, reduced uptake of ^{3}H-thymidine was observed following incubation of human peripheral blood cultures with

1,4-diamino-2-nitrobenzene (purity, 97%) for 48 or 72 h at concentrations of 25, 50 or 100 μg/ml water (Smith *et al.*, 1976).

The oral LD_{50} of the compound in oil-in-water suspension was 3080 mg/kg bw in Charles River CD rats; the intraperitoneal LD_{50} in dimethyl sulfoxide was 348 mg/kg bw in rats (Burnett *et al.*, 1977). The oral LD_{50} of the compound in water was 2100 mg/kg bw in male Wistar rats (Gloxhuber *et al.*, 1972). The intraperitoneal LD_{50} of the compound in CFW mice was reported to be 214 mg/kg bw (Mikstacki, 1985).

During a 13-week study, groups of 10 Wistar rats of each sex were fed diets containing 500 mg/kg 1,4-diamino-2-nitrobenzene [purity unspecified] to give a calculated daily intake of 30–50 mg/kg bw. No change was found in body weight, blood or urine parameters or in the histological appearance of a range of tissues in comparison with controls (Gloxhuber *et al.*, 1972).

In the chronic feeding study described on pp. 187–188, no significant compound-related non-neoplastic lesion or toxic effect was observed in rats or mice when compared with controls (US National Cancer Institute, 1979).

1,4-Diamino-2-nitrobenzene was present at low concentrations in an oxidative hair colouring formulation evaluated in a 13-week study of dermal toxicity in rabbits (Burnett *et al.*, 1976; 1.1%), in a semi-permanent formulation tested in a two-year feeding study in dogs (Wernick *et al.*, 1975, 0.24%) and in a 20-month study of dermal toxicity in mice (Jacobs *et al.*, 1984, 0.85%), described in detail on p. 97. No treatment-related adverse effect was detected. [The Working Group noted that the dose of each component of the formulations was very low and unlikely to have been toxic.]

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

1,4-Diamino-2-nitrobenzene was present at low concentrations in semi-permanent hair colouring formulations evaluated in a study of fertility and reproductive performance in rats and in studies of teratogenesis in rats and rabbits (Wernick *et al.*, 1975, 0.24%; see p. 99) and in an oxidative formulation evaluated in a two-generation study of reproduction (Burnett & Goldenthal, 1988, 1.1%) and in a study of teratogenesis in rats (Burnett *et al.*, 1976, 1.1%) (see p. 100). No treatment-related adverse effect was detected. [The Working Group noted that the dose of each component of the formulations was very low and unlikely to have been toxic.]

1,4-Diamino-2-nitrobenzene [purity unspecified] in sterile distilled water was injected subcutaneously into groups of 25–69 pregnant CD-1 mice on gestation days 6–15 at doses of 0, 32, 64, 128, 160, 192, 224 or 256 mg/kg per day (Marks *et al.*, 1981). Maternal body weight gain during gestation days 1–10 was significantly reduced in all treated groups, and doses of 128 mg/kg per day and above significantly reduced maternal weight gain throughout pregnancy. Maternal mortality occurred at doses of 224 and 256 mg/kg per day. The average

number of implants per litter was significantly reduced with 32, 128 and 160 mg/kg per day, but not at higher doses. The frequency of resorptions was significantly increased at 224 and 256 mg/kg per day, but the average number of live fetuses per litter was significantly reduced only with 32, 128 and 256 mg/kg per day. Average fetal body weights were significantly reduced with 128 mg/kg per day and above, and the number of stunted fetuses was significantly increased with 224 and 256 mg/kg per day. The percentage of malformed fetuses (cleft palate, fused ribs, bilateral open eye) was significantly increased at doses of 160 mg/kg per day and above. Under the conditions of this study, 1,4-diamino-2-nitrobenzene was developmentally toxic to CD-1 mice at doses that were also toxic to pregnant dams.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Tables 1 and 2 and Appendices 1 and 2)

1,4-Diamino-2-nitrobenzene was mutagenic to *Salmonella typhimurium*, to *Escherichia coli* and at the *tk* locus in mouse lymphoma L5178Y cells. It was neither mutagenic to *Neurospora crassa* nor recombinogenic in *Saccharomyces cerevisiae*. 1,4-Diamino-2-nitrobenzene induced dominant lethal mutation and chromosomal aberrations in germ cells of *Drosophila melanogaster* (abstract).

Unscheduled DNA synthesis was not induced in primary cultures of rat hepatocytes, whereas an extremely low dose was reported to do so in HeLa cells. Sister chromatid exchange was induced by 1,4-diamino-2-nitrobenzene in cultured Chinese hamster ovary cells, and it induced structural chromosomal aberrations *in vitro* in Chinese hamster cells and in human lymphocytes. It enhanced morphological transformation of primary Syrian hamster embryo cells, BALB/c 3T3 and C3H/10T½ mouse cells and enhanced Moloney mouse sarcoma–leukaemia virus complex induction of transformation of mouse C3H2K cells.

1,4-Diamino-2-nitrobenzene did not induce sister chromatid exchange in bone-marrow cells of Chinese hamsters treated *in vivo* orally or intraperitoneally. In mice dosed intraperitoneally, it did not induce chromosomal aberrations in bone-marrow cells or in previously injected Ehrlich ascites tumour cells. The dye was also inactive in inducing micronuclei in polychromatic erythrocytes of rat bone marrow after treatment by gavage. No dominant lethal mutation was observed in rats following intraperitoneal treatment.

The addition of hydrogen peroxide to 1,4-diamino-2-nitrobenzene had inconsistent effects upon mutagenic responses in *S. typhimurium* TA98 (Yoshikawa *et al.*, 1976, 1977).

1,2,4-Triaminobenzene, a metabolite of 1,4-diamino-2-nitrobenzene, was mutagenic to bacteria but did not induce sperm-head abnormality in mice after intraperitoneal injection.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,4-Diamino-2-nitrobenzene is used in permanent and semi-permanent hair dye formulations and for dyeing fur.

Table 1. Genetic and related effects of 1,4-diamino-2-nitrobenzene

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	25.0000	Byeon *et al.* (1975)
SAO, *Salmonella typhimurium* TA100, reverse mutation	+	0	0.0000	McMahon *et al.* (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	50.0000	de Giovanni-Donnelly (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	5.0100	Probst *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50.0000	Gentile *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50.0000	Zeiger *et al.* (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	-	25.0000	Byeon *et al.* (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	(+)	50.0000	Zeiger *et al.* (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	0	+	50.0000	Shahin *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	+	10.0000	Ames *et al.* (1975)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	2.5000	Searle *et al.* (1975)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	6.2500	Byeon *et al.* (1975)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	+	250.0000	Venitt & Searle (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	25.0000	Garner & Nutman (1977)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	0	25.0000	Ammenheuser & Warren (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	10.0000	de Giovanni-Donnelly (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	0	5.0100	Probst *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	+	50.0000	Shahin *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	25.0000	Byeon *et al.* (1975)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	25.0000	Yoshikawa *et al.* (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	5.0000	Dunkel & Simmon (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	20.0000	de Giovanni-Donnelly (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	0	0.0000	Ishidate *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	0	5.0100	Probst *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	5.0000	Gentile *et al.* (1987)

Table 1 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	5.0000	Zeiger *et al.* (1988)
SAS, *Salmonella typhimurium* TA97, reverse mutation	0	+	50.0000	Shahin *et al.* (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	17.0000	Zeiger *et al.* (1988)
SCG, *Saccharomyces cerevisiae* D4, *trp5* conversion	–	0	0.0000	Mayer & Goin (1980)
SCH, *Saccharomyces cerevisiae* D3, *ade2* mitotic recombination	–	–	500.0000	Mayer & Goin (1980)
NCR, *Neurospora crassa*, reverse mutation	–	0	400.0000	Ong (1978)
DMC, *Drosophila melanogaster*, chromosomal aberrations in germ cells	+		1000.0000, 24 h	Laethem & Wu (1985); abstr.
DML, *Drosophila melanogaster*, dominant lethal mutation	+		1000.0000	Laethem & Wu (1985); abstr.
URP, Unscheduled DNA synthesis, rat primary hepatocytes	(+)	0	100.0000	Williams *et al.* (1982)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–	0	153.0000	Probst *et al.* (1981)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	+	0	25.0000	Palmer *et al.* (1977)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus	+	0	50.0000	Oberly *et al.* (1984)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	0	15.0000	Perry & Searle (1977)
CIC, Chromosomal aberrations, Chinese hamster prostate cells *in vitro*	+	0	25.0000	Kirkland & Venitt (1976)
CIC, Chromosomal aberrations, Chinese hamster lung fibroblasts *in vitro*	+	0	0.0000	Ishidate & Odashima (1977); Ishidate *et al.* (1981)
	+	0	20.0000	
CIT, Chromosomal aberrations, mouse C3H/10T½ fibroblasts *in vitro*	+	0	1.5300	Benedict (1976)
TBM, Cell transformation, BALB/c 3T3 mouse cells	+	0	0.0000	Sivak & Tu (1985)
TCM, Cell transformation, C3H/10T½ mouse cells	+	0	1.5300	Benedict (1976)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	0	0.5000	Pienta & Kawalek (1981)
TEV, Cell transformation, Moloney sarcoma virus in mouse C3H2K cells	–	0	0.0000	Yoshikura *et al.* (1979)
UHT, Unscheduled DNA synthesis, HeLa cells	+	0	0.0150	Martin *et al.* (1978)

Table 1 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	50.0000	Searle *et al.* (1975)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	-		300.0000 ip	Neal & Probst (1983)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	-		500.0000 po	Neal & Probst (1983)
MVR, Micronucleus test, CFY rats *in vivo*	-		1000.0000 po × 2	Hossack & Richardson (1977)
CBA, Chromosomal aberrations, CFW mouse bone-marrow cells *in vivo*	-		107.0000 ip	Mikstacki (1985)
CBA, Chromosomal aberrations, mouse Ehrlich ascites tumour cells *in vivo*	-		0.0000	Bogajewski & Bogajewska (1982); abstr.
CVA, Chromosomal aberrations, mouse Ehrlich ascites tumour cells *in vivo*	-		107.0000 ip	Mikstacki (1985)
DLR, Dominant lethal mutation, CD rats	-		20.0000 ip × 24	Burnett *et al.* (1977)
DLR, Dominant lethal mutation, Holtzman rats	-		40.0000 ip × 30	Sheu & Green (1979)

+, positive; (+), weakly positive; -, negative; 0, not tested

[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw; 0.0000, not given

Table 2. Genetic and related effects of 1,2,4-triaminobenzene

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAO, *Salmonella typhimurium* TA100, reverse mutation	+	0	19.0000	Mitchell (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	19.0000[b]	Mitchell (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	+	25.0000	Garner & Nutman (1977)
SPM, sperm morphology, (CBA × BALB/c)F_1 mouse *in vivo*	-		25 × 5 ip	Topham (1980)

+, positive; (+), weakly positive; -, negative; 0, not tested
[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw
[b]S9 was detoxifying

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,4-Diamino-2-nitrobenzene was tested for carcinogenicity by oral administration in the diet in one study in mice and in one study in rats. An increased incidence of liver-cell tumours was observed in female mice. No increase in the incidence of tumours was observed in male mice or in rats.

5.4 Other relevant data

1,4-Diamino-2-nitrobenzene induced gene mutation in bacteria and in cultured mammalian cells. It did not induce gene mutation, mitotic crossing over or gene conversion in yeasts. It induced chromosomal aberrations, sister chromatid exchange and cell transformation in cultured mammalian cells and chromosomal aberrations in human lymphocytes *in vitro*. Equivocal responses were obtained for DNA damage induction in cultured rodent cells.

There was no evidence for induction of sister chromatid exchange, micronuclei, chromosomal aberrations or dominant lethal mutation in rodents dosed *in vivo*.

1,2,4-Triaminobenzene, a metabolite of 1,4-diamino-2-nitrobenzene, was mutagenic to bacteria.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of 1,4-diamino-2-nitrobenzene.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,4-diamino-2-nitrobenzene.

Overall evaluation

1,4-Diamino-2-nitrobenzene *is not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Aldrich Chemical Co. (1992) *Aldrich Catalog/Handbook of Fine Chemicals 1992–1993*, Milwaukee, WI, p. 931

Ames, B.N., Kammen, H.O. & Yamasaki, E. (1975) Hair dyes are mutagenic: identification of a variety of mutagenic ingredients. *Proc. natl Acad Sci. USA*, **72**, 2423–2427

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[1]For definition of the italicized terms, see Preamble, pp. 26–30.

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